

# The Influence of Air Temperature and Sunlight Intensity on Mate-Locating Behavior of *Pieris rapae crucivora*

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**ABSTRACT-** It is considered that the mate-locating behaviors of ectothermic insects are constrained by the ambient thermal conditions, since the flight ability depends on the body temperature. However, since ecological factors also influence the mating behaviors, the flight ability would not necessarily determine the time schedule of male mate-locating flight. To reveal how the ambient thermal conditions influence the mating behaviors, we investigated the association of air temperature and sunlight intensity with the diurnal schedule of female-searching behavior in male *P. rapae crucivora*. In the early morning, the proportion of female-searching males to basking males increased as the air temperature and sunlight intensity increased. The air temperature was also associated significantly with the diurnal schedule of male female-search on 7 of 8 observation days. The number of female-searching males reached the peak when the air temperature was between 24 and 29°C. On the other hand, the sunlight intensity did not account for the diurnal schedule of male female-search. The associations between the sunlight intensity and male female-search were not consistent among different days. When the air temperature was roughly constant, however, the sunlight intensity was correlated significantly with male activity of female-search. The regression of male activity of female-search as a function of sunlight intensity was improved, when it was assumed that the sunlight intensity influenced male behaviors with a delay of 1 to 4 minutes, and when it was assumed that the mean intensity of sunlight for 1 to 9 minutes influenced male behaviors.

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## INTRODUCTION

Most male reproductive effort is devoted to securing mates. Especially in those species lacking parental care, a male's reproductive success is largely determined by the number of females he secures. This causes competition among males for access to females. Such intense competition is believed to produce different strategies to secure mates, including territorial behavior, attraction of females by signaling, active search and so on. Thomhill and Alcock (1983) argued that scramble competition occurs when male territoriality is less advantageous, since many males compete for females so intensely that they can afford little female guarding, or when it is difficult to predict the availability of females. In such cases the most important factor affecting mate securing may be the ability to locate mates as quickly and steadily as possible (Odendaal *et al.*, 1985).

Scramble competition occurs in some lepidopterans (Rutowski, 1991). Under the scramble competition, the reproductive success of male butterflies is dependent on their diurnal schedule of mate-location (Iwasa & Obara, 1989). Rutowski *et al.* (1996) reported that male *Asterocampa leilia*

(Nymphalidae) perform the mate-locating behaviors more intensely when the receptive females are more available. However, the behaviors of ectothermic insects are constrained by ambient thermal conditions (Heinrich, 1996). The constraint is crucial for the flight behaviors, since the performance of thoracic flight muscle is dependent on the thoracic temperature, which in turn depends on the ambient thermal conditions (May, 1983; Marden, 1995). It follows that the diurnal schedule of mate-location should be also restricted by the ambient thermal conditions.

It is also theoretically suggested that male *Pieris rapae crucivora* (Pieridae) adjust the diurnal schedule of female-searching flight to female availability (Iwasa and Obara, 1989), which is supported empirically (Hirota and Obara, 2000). However, the influence of ambient thermal conditions on the diurnal schedule of male female-search has not been evaluated quantitatively. Ohsaki (1986) suggested that the body temperature from 28 to 32°C was suitable for flight behaviors in *P. rapae crucivora*. Rutowski *et al.* (1996), however, reported that male butterflies did not always perform the mate-locating behavior even when the ambient thermal conditions were suitable for flight behaviors in *A. leilia*. It follows that the time schedule of general flight behavior does not necessarily represent that of mate-locating flight. This was consistent with our previous observation that

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the diurnal schedule of flower-visiting behavior was different from that of female-searching behavior (Hirota and Obara, 2000). Therefore, it remains to be quantified how the ambient thermal conditions explain the diurnal schedule of male female-search.

In the present study we observed the male mate-locating behavior throughout the day under different meteorological conditions, and analyzed how it is affected by the air temperature and the sunlight intensity.

## METHODS

### General method

*P. rapae crucivora* used for the observation were derived from the wild population of the experimental farm of Tokyo University of Agriculture and Technology, in Fuchu, Tokyo. In a corner of the experimental farm, the cabbages, *Brassica oleracea*, one of host plants of *P. rapae crucivora*, were cultivated in the field of 20 × 25 m. Buckwheat (*Fagopyrum esculentum*) was planted around the cabbage field and its flowers served as the nectar source for the butterflies. We released more than 80 male butterflies to the cabbage field, and counted the males searching for females throughout a day. Most of the released males would remain in the cabbage field, since male *P. rapae* do not disperse far (Ohsaki, 1982). The female-searching behavior of males was characterized by slow zigzag flight of less than ca. 15 cm above the leaves of *B. oleracea* (Obara, 1964; Ohtani, 1985; Hirota and Obara, 2000). Males were also frequently observed flying under the leaves of *B. oleracea* for mate-location. We transected the cabbage field and counted the males searching for females. Counting the males in the whole cabbage field was carried out within one minute. Immediately after counting the males, air temperature and sunlight intensity were recorded with a TNA-120 (TASCO Japan Co., Ltd.), and a digital illuminometer T-1M (Minolta Camera Co., Ltd.), respectively.

Stepwise polynomial regression was carried out to analyze the influence of air temperature and sunlight intensity on male female-search (Zar, 1999). The forward selection procedure was used to determine the maximum power of the polynomial that had statistical significance. The normality of data was confirmed with the D'Agostino-Pearson test (Zar, 1999). The proportional data was normalized with arcsine transformation.

### Observation 1: Comparison of the number of basking males

The female-searching males were counted every 3 min. from 6:00 to 8:00 a.m. and every 10 min. thereafter until dusk on 1 June, 1983. At the same time, the males basking in the sun were also counted to determine how their female-searching activities were constrained by low body temperature. The basking posture of *Pieris* butterflies is characterized by positioning the dorsal surface of the thorax towards the sunlight with the wings opened (Kingsolver, 1985; Ohtani, 1985). The proportion of the number of female-searching males to that of active males including both female-searching and basking males was regressed on the air temperature or the sunlight intensity.

### Observation 2: Diurnal schedule

To evaluate the influence of meteorological factors on the diurnal schedule of male female-search, the female-searching males were counted every 30 min. from dawn to dusk on the following eight days: May 30, June 1, July 14 in 1983 and July 12, July 14, July 15, August 10, August 11 in 1984.

### Observation 3: Influence of sunlight intensity

To evaluate how the sunlight intensity influences the male female-search, the air temperature should be kept constant. This may be possible if we carry out observation on the male female-search for a short time, because the air temperature is expected to remain constant for a short time even if the sunlight intensity fluctuates more quickly. We therefore counted the female-searching males every 1 min. from 10:00 to 14:00 on June 6 in 1983, selected a period when the air temperature was relatively constant and carried out the regression analysis on the selected data.

## RESULTS

### Observation 1: Behaviors at lower air temperature

In the early morning when the air temperature was lower, male butterflies moved to sunny areas and performed the basking behavior before they started the female-search flight (Fig. 1a). Some male butterflies started the female-searching flight around the time when the number of basking males reached the peak. The number of basking males decreased as that of female-searching males increased, and no basking behavior was observed after 8:50 when the air temperature was 27.5°C. The proportion of basking males to active males was correlated quadratically with air temperature (Fig. 1b,  $n=40$  periods,  $R^2=0.829$ ,  $p<0.001$ ) and linearly with sunlight intensity (Fig. 1c,  $n=40$ ,  $R^2=0.748$ ,  $p<0.001$ ). That is, more males performed the basking behavior than female-searching flight at lower air temperature and under lower sunlight intensity. Multiple regression was not carried out because the air temperature and the sunlight intensity correlated significantly ( $R=0.891$ ,  $p<0.001$ ).

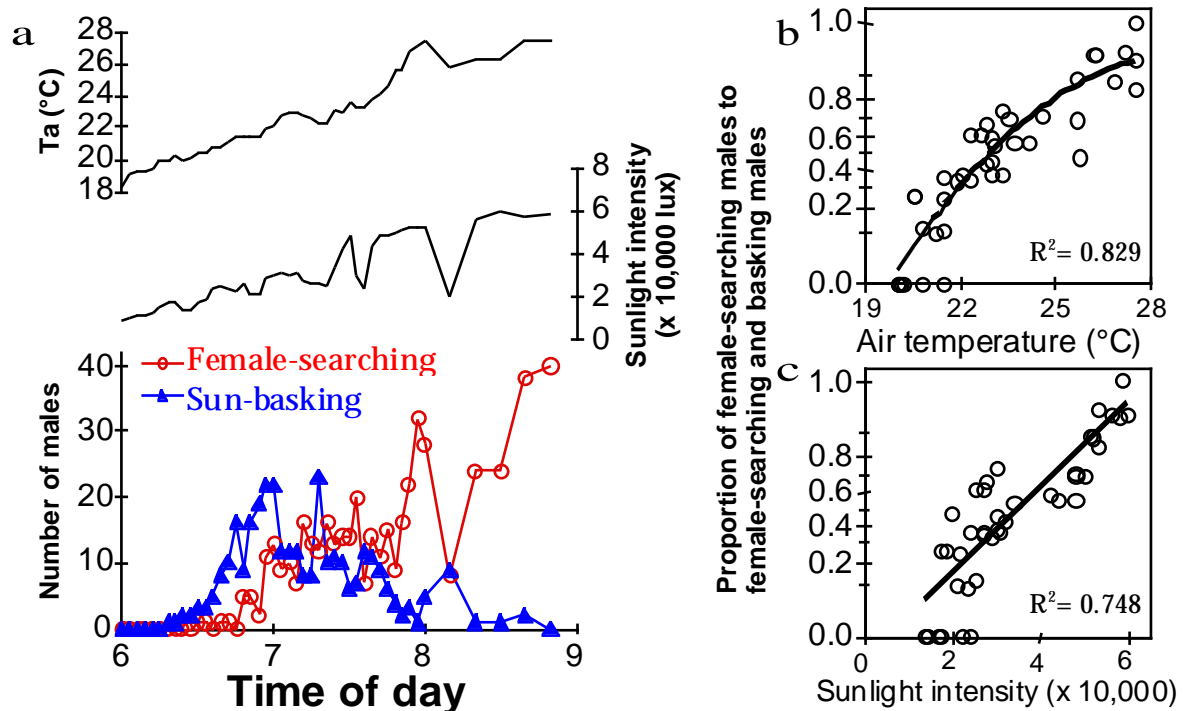
### Observation 2: Diurnal schedule

On 7 of 8 days, the air temperature explained successfully the diurnal change of the number of female-searching males (Fig. 2). Stepwise polynomial regression showed that quadratic regression was significant on the data on July 14, 1983 and August 10, 1984 (Fig. 2c, g, respectively), and that cubic regression was significant on the data on June 1, 1983 and July 12, July 14, July 15, August 11, 1984 (Fig. 2b, d, e, f, h, respectively). On those seven days the number of female-searching males reached the peak at an intermediate air temperature, although the temperature at the peak ranged from 24 to 29 °C.

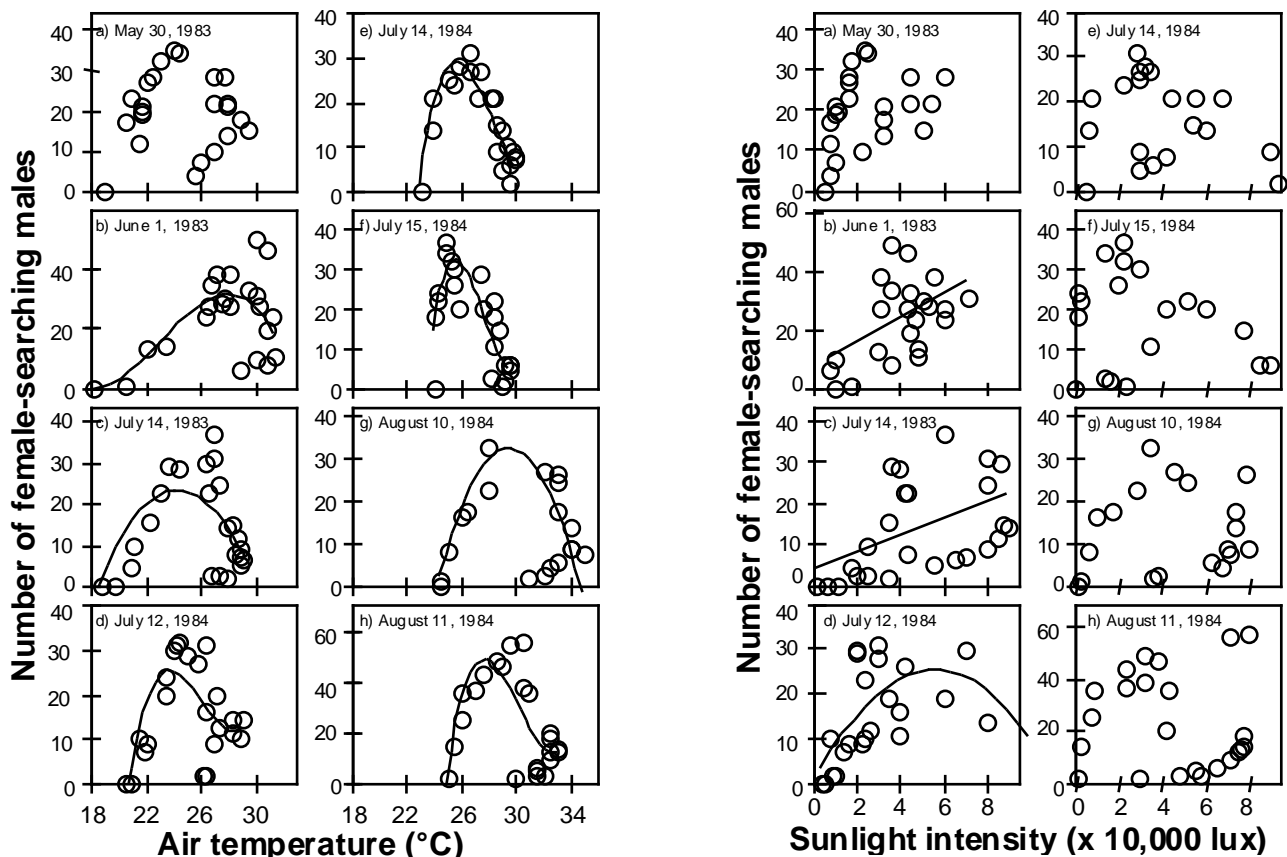
The sunlight intensity failed to explain well the diurnal change of the number of female-searching males, although there was a significant association on June 1, July 14 1983 and July 12, 1984 (Fig. 3b, c, d, respectively). The linear regression was significant for the data on June 1, July 14, 1983, whereas the quadratic regression was significant for those on July 12, 1984. Multiple regression was not carried out because the air temperature and the sunlight intensity correlated significantly for all days ( $R>0.65$ ,  $p<0.05$ ) except for June 1, 1983 ( $R=0.39$ ,  $p=0.07$ ).

### Observation 3: Sunlight intensity and female-search

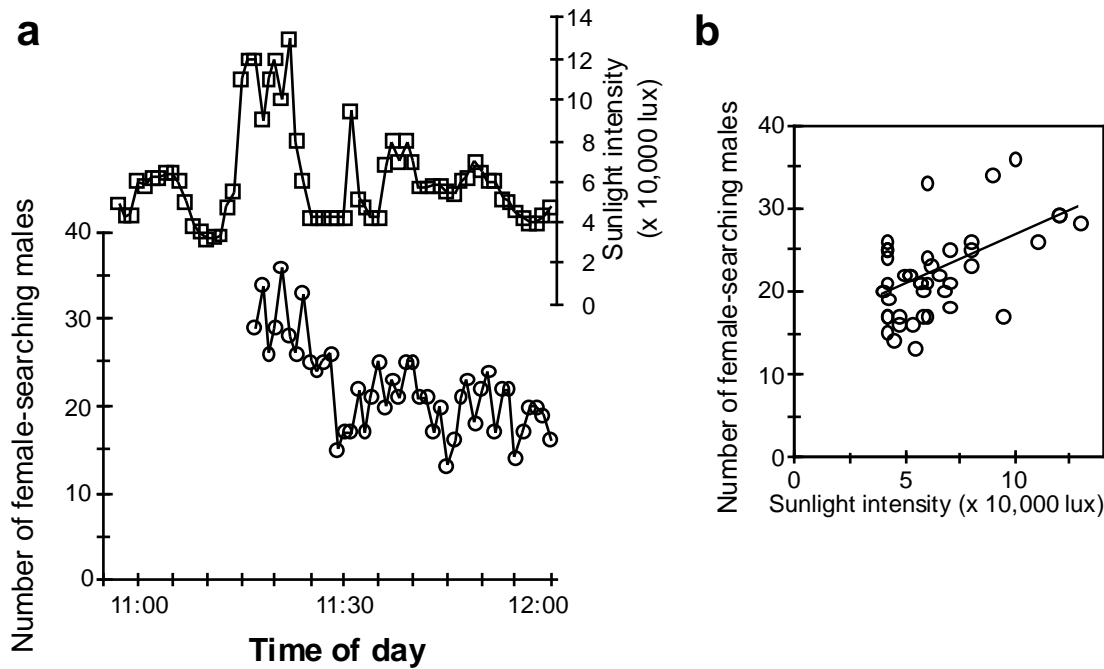
To eliminate the influence of air temperature, we analyzed the data obtained by the short period of observation



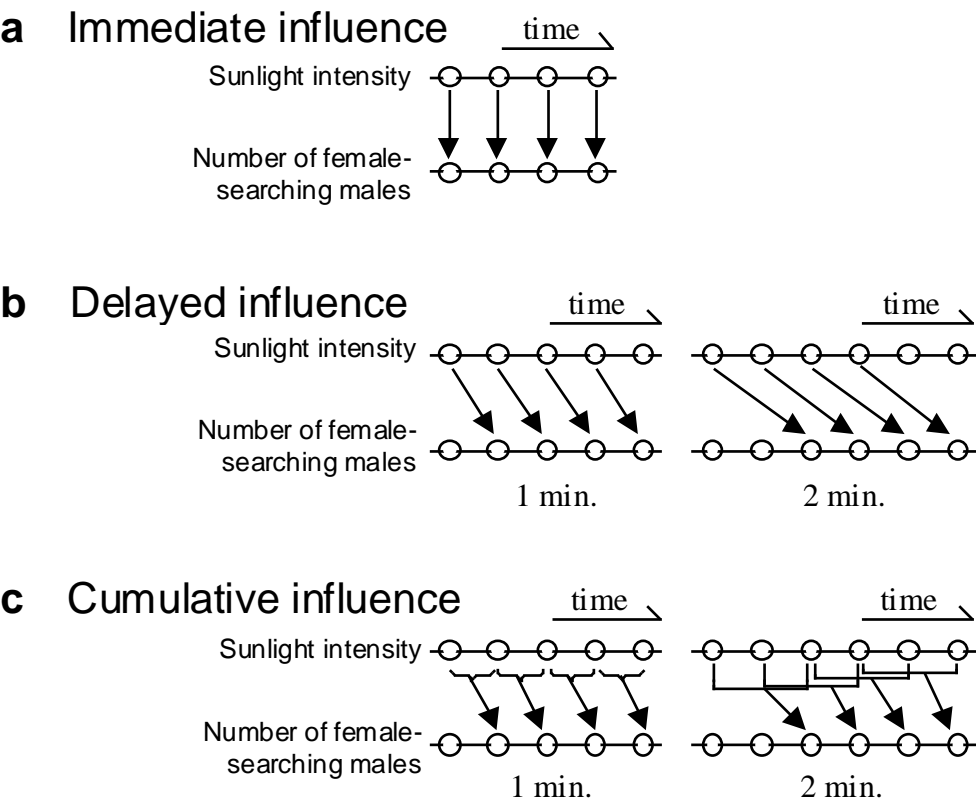
**Fig. 1.** The influence of air temperature and sunlight intensity on male basking and female-searching behaviors. a: the number of males performing sun-basking (filled triangles and blue line) and female-search (open circles and red line), with the air temperature and sunlight intensity. b, c: The proportion of female-searching males to active males (basking males + female-searching males) was regressed on the air temperature (b) and sunlight intensity (c). The Y-axis is graduated in the arcsine scale ( $y' = \arcsin \sqrt{y}$ ).



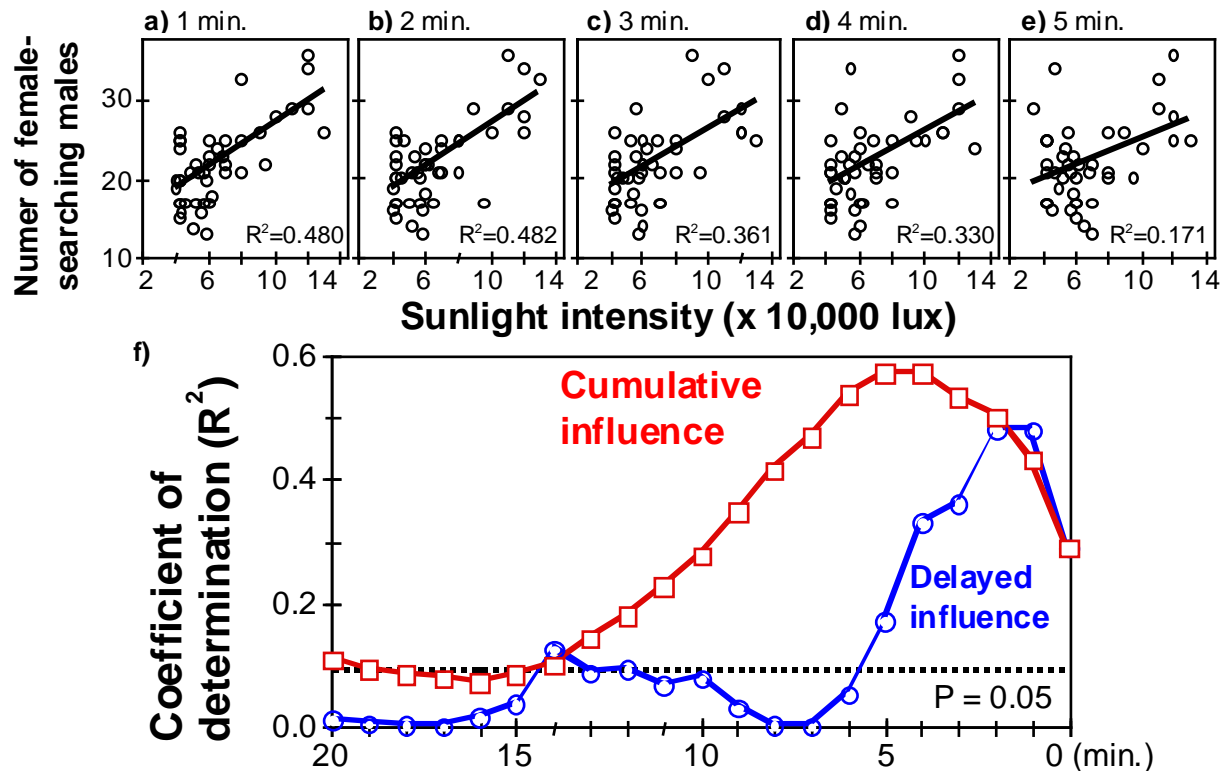
**Fig. 2.** The influence of air temperature on the number of female-searching males.



**Fig. 4.** The influence of sunlight intensity on male activity of female-search. a: The number of female-searching males at 11:17-12:00 and the sunlight intensity at 10:57-12:00. b: The association between the number of female-searching males and the sunlight intensity at 11:17-12:00.



**Fig. 5.** Conceptual models for the influence of sunlight intensity on the male female-search. a: The sunlight intensity would influence the number of female-searching males immediately. b: The sunlight intensity would influence the number of female-searching males with an interval of some minutes. c: The number of female-searching males would be influenced by the mean sunlight intensity for some minutes before counting the female-searching males.



**Fig. 6.** The influence of sunlight intensity on male activity of female-search. a-e: The number of female-searching males was regressed as a function of the sunlight intensity at 1-5 min. before counting males. f: The number of female-searching males was regressed either as a function of the sunlight intensity at 1-20 min. before counting males (circles and blue line), or as that of the mean intensity of sunlight for 1-20 min. before counting males (squares and red line). The Y-axis represents the coefficient of determination resulting from the linear regression analysis. The dotted line represents the critical value of the significant probability, 0.05. The points appearing above the dotted line represent a significant association.

from 11:17 to 12:00, when the air temperature remained roughly constant between 26.0 and 27.5°C, while the sunlight intensity changed largely from 40,000 to 130,000 lux (Fig. 4a). The sunlight intensity was correlated linearly with the number of female-searching males (Fig. 4b;  $n=44$  periods,  $R^2=0.290$ ,  $p<0.001$ ). In the course of observation, however, it was noticed that there was a lag between the change in the sunlight intensity and the male behavioral response to it. Taking this lag into consideration, we performed the regression analysis between the number of female-searching males and the sunlight intensity at 1 to 20 minutes before counting males (Fig. 5b). For the lag of 1 to 5 min., there was a significant linear regression ( $R^2=0.480$ , 0.482, 0.361, 0.330, 0.171, respectively; Fig. 6a-e). Although the linear regression was also significant for the lags of 12 and 14 min., the coefficients of determination ( $R^2$ ) were not so large (Fig. 6f). The maximum coefficient of determination was obtained for the 2 min. lag.

If the sunlight intensity has a cumulative influence on male behaviors rather than a delayed influence, the mean sunlight intensity for some minutes before counting the female-searching males would explain well the number of female-searching males (Fig. 5c). The mean sunlight intensity for 1-20 min. before counting female-searching males was regressed on the number of female-searching males. The

mean sunlight intensity for 1-14 min. explained significantly the female-searching males (Fig. 6f). Although the linear regression was also significant on the mean sunlight intensity for 19-20 min., the coefficients of determination were not so large.

## DISCUSSION

In the early morning when the body temperature was relatively low (Ohsaki, 1986), the activity of male female-searching flight was significantly associated with the air temperature and the sunlight intensity (Fig. 1). The number of basking males decreased and that of female-searching males increased as the air temperature and sunlight intensity increased. This showed a relatively greater number of basking males in the early morning, since their body temperature was not high enough for flight behaviors (Kingslover, 1985; Ohsaki, 1986).

The air temperature was also associated significantly with the diurnal schedule of male female-search on 7 of 8 observation days (Fig. 2b-h). On those 7 days the number of female-searching males reached the peak at intermediate air temperatures. This is consistent with the report that the performance of thoracic flight muscle declines as the thoracic temperature deviates from the optimum (May, 1983; Marden,

1995). However, the air temperatures at which male female-search reached the peak ranged so widely from 24 to 29°C that it was difficult for a common regression model to predict the diurnal schedules of male female-search throughout different days.

The sunlight intensity failed to account for the diurnal schedule of male female-search (Fig. 3). On 5 of 8 observation days there was no significant association between the sunlight intensity and the number of female-searching males (Fig. 3a, e-h), except for the linear regression on 2 of 8 days (Fig. 3b, c) and the quadratic regression on 1 of 8 days (Fig. 3d). Why does the sunlight intensity fail to account for the diurnal schedule of male female-search? One possibility is that the sunlight might not be an important heat source in the later morning. When the air temperature was over 28 °C, males could easily attain the body temperature sufficient for flight, which was suggested to range from 28 to 32 °C (Ohsaki, 1986). It follows that the air temperature influences more strongly the diurnal schedule of male female-search, so the influence of sunlight intensity would be less detectable. This coincides with the result that the sunlight intensity was correlated significantly with the activity of male female-search when the air temperature was relatively constant (Fig. 4b).

The regression of sunlight intensity on male activity of female-search was improved by assuming the delay of 1 to 4 min. between the change of sunlight intensity and male response to it (Fig. 6b-d). The coefficient of determination was increased further when the mean intensity of sunlight for 1 to 9 min. before counting the female-searching males was used as an independent variable (Fig. 6f). Why does the mean intensity of sunlight account well for male activity of female-search? One possibility is that it takes some time for sunlight to raise the body temperature. However, it should not take so long for males to achieve the body temperature of 28°C or higher (Ohsaki, 1986), which might be the lower limit of body temperature sufficient to fly, since in the present observations the air temperature ranged from 26.0 to 27.5 °C. Another possibility may concern the visual cues for mate recognition which would be influenced by sunlight intensity. This is because the male *P. rapae crucivora* recognizes a conspecific female as a mate by her wing color which includes ultraviolet color as an essential component (Obara and Hidaka, 1968). It is therefore likely that males could not discriminate females from males effectively when the intensity of ultraviolet rays is low. It follows that males should perform other behaviors (e.g. flower-visiting) rather than female-searching when the sunlight intensity remains relatively low for a while. Since both possibilities are not exclusive, further studies remain to be done to determine how closely the factors affect the male female-search. It should be noted, however, that the present study suggests that the correlation between sunlight intensity and male activity of female-search can not be explained successfully without taking into account the delayed influence or cumulative influence of sunlight intensity.

The present study revealed distinct associations between two meteorological factors, air temperature and sunlight intensity, and the male activity of female-search either in the early morning when the thermal restriction was obvious or under relatively constant air temperature. On the other hand, those meteorological factors failed to explain the variance in diurnal schedules of male female-search. In addition to thermal factors, such factors as predation pressure and availability of receptive females should be involved in the diurnal schedule of male mate-location (Iwasa and Obara, 1989; Rutowski *et al.*, 1996). If the predation pressure was strong, males would refrain from searching for females even when thermal conditions were appropriate for flight (Begon *et al.*, 1996). *P. rapae crucivora*, however, did not appear to be under strong predation pressure. Kingsolver (1987) also reported that *Pieris* butterflies were less susceptible to predation while thermal conditions allow them effective flight. This suggests that the effect of predation does not outweigh that of thermal conditions.

It is likely that the temporal distribution of newly-emerged females which may be most valuable to males (Rutowski, 1991) would vary on different days, since the timing of female eclosion from pupae is dependent on the ambient temperature. The female post-emergence behaviors also depend on the ambient temperature, thereby influencing the female availability to males (Hirota *et al.*, 2001). Further studies are necessary to evaluate how the variance of female availability over days is involved in the variance of diurnal schedule of male female-search.

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